Penetration of the Laser Light Into the Skin In Vitro

H. Kolárová, PhD,[1]* D. Ditrichová, MD,[2] and J. Wagner, PhD[3]

[1]*Center of Molecular Biology and Medicine, Department of Medical Biophysics, Medical Faculty, Palacký University, 77515 Olomouc, Czech Republic*
[2]*Department of Dermatology, Medical Faculty, Palacký University, 77520 Olomouc, Czech Republic*
[3]*Department of Optics, Faculty of Science, Palacký University, 77200 Olomouc, Czech Republic*

Background and Objective: **Knowledge of the optical parameters of the skin is important for all kinds of phototherapy. We analyzed penetration of laser light and proved different optical properties of in vitro specimens of normal skin and granular tissue from skin ulcers.**
Study Design/Materials and Methods: **An He-Ne laser (λ = 632.8 nm, output 50 mW) and a semiconductor laser (λ = 675 nm, output 21 mW) were used. The distribution of laser radiation was detected by a CCD camera and evaluated by the image analysis software DIPS.**
Results: **Transmittance in granular tissue was about 2.5 times higher than that in normal skin. In the thickest skin sample (2 cm), approximately 0.3% of He-Ne laser and 2.1% of semiconductor laser light penetrated.**
Conclusions: **The results demonstrate the percentage of incident light penetrating the individual skin layers in different localizations on the skin surface, which is a decisive factor for the selection of the radiation dose. Lasers Surg. Med. 24:231–235, 1999.** **© 1999 Wiley-Liss, Inc.**

Key words: He-Ne laser; semiconductor laser; skin; transmittance

INTRODUCTION

Electromagnetic radiation of different wavelengths, including laser radiation, is commonly used in dermatology, which accounts for the attention paid to practical aspects of the interaction between radiation and human skin and to the problem of selective absorption and depth of radiation penetration into the skin. As a wavelength increases into the visible and near-infrared optical region of the spectrum, radiation penetrates more deeply into the skin. However, only few experimental studies on this subject have been reported [1–3]. The aim of the present study was to verify these parameters with regard to the importance of having accurate information about the optical parameters of the skin for laser therapy and for phototherapy in general.

Photodynamic therapy, which uses laser light in the visible wavelength region, in combination with tumor-sensitizing drugs such as different porphyrins, chlorins, or phthalocyanine-containing substances, is an example of a new, nonthermal laser interaction with biological tissue. The method has been applied in the treatment of different types of tumors, including skin malignancies [4–6].

Optimizing such treatments requires quantitative knowledge of light propagation in the skin related to the optical properties of the individual

Contract grant sponsor: Ministry of Education of the Czech Republic; Contract grant number: VS 96154.

*Correspondence to: H. Kolárová, Center of Molecular Biology and Medicine, Department of Medical Biophysics, Medical Faculty, Palacký University, Hnevotínská 3, 77515 Olomouc, Czech Republic. E-mail: kol@tunw.upol.cz

Accepted 23 October 1998

skin layers. The present study of skin optics concerns the penetration of laser light of different wavelengths through the individual skin layers and granular tissue.

MATERIAL AND METHODS

In this study, an He-Ne laser (LA 1001, Metra Blansko, Czech Republic; λ = 632.8 nm, output 50 mW) and a semiconductor laser (Med 140, Lasotronic, Switzerland; λ = 675 nm, output 21 mW) were used. The output meter for the He-Ne laser and photodetector are accessories of the laser produced by Metra Blansko. The output meter for measurement of the power of the semiconductor laser Field Master (Coherent, USA) was used.

Distribution of the irradiance of laser radiation was detected with a CCD camera and evaluated by the image analysis software DIPS, version 4.0.

The skin samples were obtained from 11 men, aged 16–40 years, undergoing plastic surgery. The specimens were treated immediately after removal and kept in a moist chamber at all times.

The skin samples removed from the plantar area, volar area of the wrist, abdominal wall, and dorsal area were measured, with and without subcutaneous fat. The (2 × 2 cm) samples were heated for 30 s in a water bath set to a temperature of 60°C, and then the epidermis was separated. In addition to specimens of normal skin, samples of granular tissue from crural ulcers were studied. The specimens were inserted between the light source and the probe with the photodetector.

RESULTS

The results from the measurement of the irradiance distribution of radiation are shown in Figure 1.

Distribution of laser irradiance is not uniform. The definition of the uniformity of radiation in the spots is given by:

$$U = \frac{I_{min}}{I_{max} + I_{min}} \qquad (1)$$

where U is the uniformity of distribution of intensity radiation, I_{mix} is the minimal intensity, and I_{max} is the maximal intensity.

The results obtained from measuring the penetration of He-Ne and semiconductor laser light into skin specimens and granular tissue are presented in Table 1. All results are the average measurement of three samples.

DISCUSSION AND CONCLUSIONS

Obtaining an accurate distribution of light in tissues presents a considerable problem in all fields of photobiology and in the application of the optical spectrum in diagnostics and therapy. The transmission of optical radiation in human skin depends on many individual factors different for each skin layer. In general, part of the light is reflected after it reaches the skin and part of the light penetrates into deeper layers, where it may be either scattered or absorbed according to the optical properties of the tissue.

Reflectance (R) is defined as follows:

$$R = \frac{I_R}{I_0} \, (\%) \qquad (2)$$

where I_R is the intensity of reflected light and I_0 is the intensity of incident light. To calculate the total reflectance of light remittance also may be comprised, i.e., reflectance caused by back scattering from deeper layers. Light transmission is similarly defined by transmittance (T):

$$T = \frac{I_T}{I_0} \, (\%) \qquad (3)$$

where I_T is the intensity of transmitted light and I_0 is the intensity of incident light.

Light absorption, particularly in homogeneous materials, is very often expressed by the absorption coefficient correlated to skin thickness and wavelength. The intensity of transmitted light can be explained by the Lambert-Beer law:

$$I_T = I_0 \cdot e^{-Kt} \qquad (4)$$

where K is the absorption coefficient and t is the thickness of the skin.

The validity of equations 2 and 3 is general. Equation 4 can be applied to the case in which absorption predominates scattering and is quite accurate only for homogeneous material. In the skin, absorption is predominant in the epidermis and upper dermis, whereas scattering by collag-

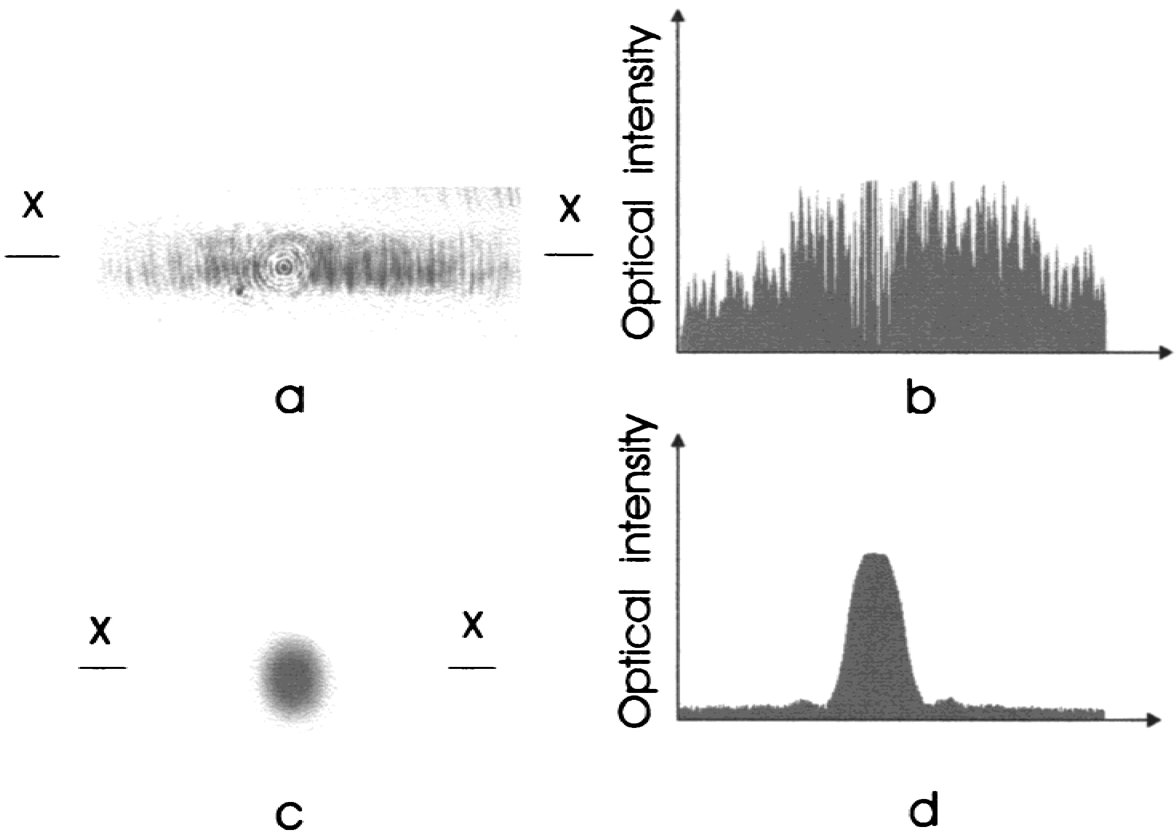

Fig. 1. Distribution of laser radiation. **a:** An area, 4 × 1 mm, irradiated by the semiconductor laser; the total power in this area was 50 mW. **b:** The relative intensity distribution of radiation by the semiconductor laser in the cutting plane X. **c:** Area irradiated by the He-Ne laser; the total power in this area was is 50 mW. **d:** Intensity distribution of radiation from the He-Ne laser in the axis beam (Gauss distribution).

enous fibers in deeper layers is predominant [7]. General mathematical modeling of multilayer materials was developed by Kubelka and has been applied to model skin by Anderson and Parrish [7] and Wan et al. [8]. Optically, the skin can be considered to consist of three distinct layers: (a) the epidermis, (b) the upper dermis containing the superficial plexus, and (c) the layer beneath the superficial plexus.

In the therapeutic application of laser radiation, it is very important to determine the doses of irradiation (or exposure) from the point of intensity and time of radiation:

$$E = \frac{P}{S}\,(W \cdot m^{-2}) \qquad (5)$$

$$D = I \cdot t\,(W \cdot s \cdot m^{-2}) \qquad (6)$$

where E is irradiance, P is the power of the laser, S is the irradiation square, D is the doses of irradiation, I is intensity, and t is time of irradiation.

In equation 6, we must calculate with nonuniformity of the distribution of intensity. Nonuniformity may be the reason for the sometimes inconsistent results that occur in some cases.

Penetration and scattering of ultraviolet (UV) light and visible light in skin very much depend on the wavelength of the emission source and the optical properties of individual skin layers. In the epidermis, the major absorbing entity in this spectral region is melanin. The wavelength of 400–600 nm is absorbed in the dermis by blood chromophores: hemoglobin, oxyhemoglobin, bili-

TABLE 1. Penetration of He-Ne Laser and Semiconductor Laser Light in Different Skin Samples In Vitro

Localization	Tissue	Thickness (mm)	Penetration (%) λ = 632.8 nm	Penetration (%) λ = 675 nm
Regio abdominis	Epidermis	0.03	80.5	86.5
	Epidermis + dermis	2.60	6.5	15.3
	Epidermis + dermis + subcutaneous fat	19.00	0.3	2.1
Regio dorsi	Epidermis	0.035	68.00	71
	Epidermis + dermis	4.1	4.8	6
Regio antebrachii anterior	Epidermis	0.024	58.0	63
	Epidermis + dermis	1.5	10.0	14
Regio femoris anterior	Epidermis	0.032	67.0	71
	Epidermis + dermis	2.2	7.2	9.8
Regio cruris anterior	Granular tissue from ulcus cruris	1.5	25.00	33

rubin, and carotene. A weak absorption by blood occurs at wavelengths of 700–1,300 nm, with a low scattering in the dermis. The transmittance of light in the epidermis for wavelengths of 250–800 nm was studied in detail by Wan et al. [8]. In the present study on the epidermis, we focused on penetration of the He-Ne laser at a wavelength of 632.8 nm and the semiconductor laser at a wavelength of 675 nm. Transmittance was 58–86.5% with regard to removal of different specimens from the skin surface. A decreased absorption in the skin in the visible spectral region is caused by a considerably lower amount of biologically important chromophores in comparison with UV radiation (melanin, DNA, urocanic acid, aromatic amino acids) [7].

Lasers are widely used in medicine because of the selective absorption of certain wavelengths in different tissue structures. For example, in dermatology, the treatment of hemangiomas, lentigines, and tattoos is enabled by the use of argon lasers, CO_2 lasers, or, recently, dye lasers. The He-Ne laser and semiconductor laser can be used for biostimulation in the therapy of poorly healing wounds of different etiology, relapsing manifestations of herpes simplex, alopecia areata, acupuncture, or examination of skin microcirculation [9–13]. The He-Ne laser can be applied in the photodynamic therapy of malignant cutaneous tumors and of metastasizing processes in combination with photosensibilizers-hematoporphyrine derivatives [14]. A disadvantage of these dyes is that they are a mixture of different, mostly unidentified compounds. Moreover, they absorb light at relatively short wavelengths that do not penetrate deeply into tissue. To circumvent these disadvantages, other dyes such as chlorins and phthalocyanines, with absorption at approximately 680 nm, have been tested [15–17].

For all these applications, accurate information about the space distribution of light in normal skin and in the pathologically changed skin is required. On the basis of the determined optical properties of the skin, parameters of radiation can be appropriately assessed, namely wavelength, source output and radiation dose, method of application, etc. For these reasons, we attempted to describe the penetration of laser light into individual skin layers from different skin localizations and to compare several optical parameters of normal skin and granular tissue. Laser radiation of wavelengths 632.8 nm and 675 nm penetrates into all skin layers. The thickest specimen was 19 mm, and approximately 0.3% of He-Ne laser light and 2.1% of semiconductor laser light penetrated this sample. In specimens without subcutis, transmittance according to localization was 4.8–10% for the He-Ne laser and 6–14% for the semiconductor laser. Granular tissue showed significantly different optical properties, and the measurement of laser light penetration was almost 2.5 times higher than the penetration in normal skin of comparable thickness because of the different histologic structure. Differences were also found among specimens of normal skin with different localizations on the skin surface. These results demonstrate the percentage of incident light penetrating the individual skin layers in different localizations on the skin surface, which is a decisive factor for selecting the radiation dose. Accurate information about light penetration in granular tissue is important for possible application of the He-Ne laser in the therapy of ulceration and nonhealing wounds of different origins.

Advancement of phototherapy and photochemotherapy is based not only on new photodynamically active substances and better sources of electromagnetic radiation (e.g., laser) but also on

greater knowledge of the optical properties of the skin and thus the possibility to influence them.

Experimentations in this field have provided some information about the first optical phase of the radiation–tissue interaction. With regard to phototherapeutic and diagnostic applications in clinical practice, this radiation–tissue interaction has to be analyzed. During these applications, the specific optical properties of every skin layer should be taken into account so that appropriate parameters of radiation can be selected.

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